Fatal Pulmonary Infection Associated with a Novel Organism, “*Parastreptomyces abscessus*”

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Actinomycetes are increasingly recognized as pathogenic in the immunocompromised host. We isolated an asporogenous, nonmotile, aerobic gram-positive rod from a transplant recipient with a fatal pulmonary infection. The pathology was similar to that associated with *Rhodococcus equi*, including intrahistiocytic localization. The organism was relatively inert in standard biochemical tests. 16S rRNA gene sequencing indicated a potentially unique organism most closely related to the genus *Streptomyces*, for which we propose the name “*Parastreptomyces abscessus*.”

CASE REPORT

A 50-year-old man with a history of chronic myelogenous leukemia presented with a new left lower lobe pulmonary mass 3 months after receiving a matched-related donor stem cell transplant.

The patient's posttransplant course was complicated by graft-versus-host disease in the gastrointestinal tract that was refractory to high-dose prednisone (2 mg/kg of body weight daily); he was subsequently treated with six doses of horse anti-thymocyte globulin (Pharmacia, Peapack, NJ) at 30 mg/kg every other day. A surveillance chest x-ray obtained 1 month later revealed a large new opacity in the left lower lobe. Computerized axial tomography of the chest confirmed the presence of a rounded, 5-cm, left lower lobe mass with indistinct borders and internal air bronchograms without cavitation; significant hilar adenopathy and calcified mediastinal lymph nodes were also noted. He reported no shortness of breath, dyspnea on exertion, or chest pain. He also denied any fevers, chills, nausea, vomiting, or night sweats. He endorsed a minimal cough at night, which was nonproductive.

He was employed as a photographer, and he denied recent travel outside of the region, pets, or exposure to farms or livestock. He had a 30-pack-year history of tobacco use (but had quit 8 years previously) and denied the use of illicit drugs. Medications included trimethoprim-sulfamethoxazole, valacyclovir, itraconazole solution, cyclosporine, and prednisone (50 mg daily).

Physical examination was unremarkable; the white blood cell (WBC) count was 1850 cells/mm³ with an absolute neutrophil count of 1200 cells/mm³. A Gram stain of bronchoalveolar lavage fluid showed occasional WBC and occasional gram-positive cocci; KOH, modified Gimenez and silver stains were

negative. Direct fluorescent antibody staining and culture for the human herpesviruses, community-acquired respiratory viruses (respiratory syncytial virus, parainfluenza viruses, and influenza virus), and *Legionella* spp. were negative. Bacterial and fungal cultures yielded approximately 20,000 colonies/ml of streptococci, 16,000 colonies/ml of coagulase-negative *Staphylococcus*, and 50,000 colonies/ml of a presumptive *Lactobacillus* species. The patient thus underwent a left lower lobectomy to establish a diagnosis. Tissue imprints from the resected lung tissue showed 4+ gram-positive rods and 4+ WBCs; pathology showed consolidated lung tissue in which macrophages filled the alveolar spaces. Numerous small gram-positive, acid-fast-negative coccobacilli were present within the macrophage cytoplasm. Based upon these findings, a presumptive diagnosis of *Rhodococcus equi* pneumonia was made.

On the same day, a surveillance blood culture (obtained 2 weeks prior to radiographic evidence of pneumonia) was reported as positive for a pleomorphic gram-positive rod.

Pending final identification of the organism, the patient was started on intravenous vancomycin and high-dose ciprofloxacin. Computerized axial tomography of the chest 1 week after biopsy showed new bilateral pulmonary opacities. Bronchoalveolar lavage showed a diffuse admixture of primarily intracellular gram-positive coccobacilli. Azithromycin, imipenem, and rifampin were added to his antibacterial coverage. Progressive respiratory failure ensued, and the patient died 3 days later. Requests for a limited autopsy were denied.

Microbiological characterization. The organism was isolated from a Bactec 9240 MycoF/Lytic fungal blood culture medium (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) and from cultures of lung tissue on routine bacterial media (5% sheep blood agar, chocolate agar, and MacConkey agar; Remel, Lenexa, KS).

Routine bacterial, fungal (Sabouraud dextrose agar; Remel), and mycobacterial (Middlebrook 7H11 agar; Remel) media were used for subculturing to determine optimal growth conditions. Determination of colonial morphology was made from 5% sheep blood agar (Remel) and tap water agar (2). A slight haze could be seen on blood and chocolate agar within

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FIG. 1. Gram stain of *Parastreptomyces abscessus* from blood culture medium, showing pleomorphic, slightly branching, gram-positive rods.

48 h. There was no growth on MacConkey agar and no growth anaerobically after 4 days. Tan colonies (2 to 4 mm in diameter) were seen on blood agar and chocolate after 4 days of incubation. There was no growth on either fungal (Sabouraud dextrose agar; Emmons) or mycobacterial (Middlebrook 7H11 agar) medium. The organism grew best in 5% CO₂ at 35°C; there was no growth at 42°C. Growth on tap water agar, inoculated directly from positive cultures obtained with lung tissue (see above), was not typical for mycobacteria, *Streptomyces* spp., or *Nocardia* spp. but was considered consistent with *Rhodococcus* spp. (coccobacilli with diphtheroidal arrangements showing no substrate or aerial mycelia).

Large and small colony types were seen on sheep blood agar after 1 week of incubation. Both were tan or buff colored, with the larger colony being slightly raised. Gram stains of the large colony showed the same pleomorphic rods, while the small colony had more coccoid forms. Gram stain morphology was determined after heat fixing smears made from 2 days of growth in either brain heart infusion broth medium or tripticase soy yeast broth. Gram stains from the blood culture medium revealed slightly branching, pleomorphic gram positive rods in clusters (Fig. 1); occasional coccoid forms were also present. Coccoid forms predominated in stains from tissue specimens (Fig. 2).

Tests for hydrolysis of xanthine, hypoxanthine, tyrosine, and casein were negative by standard methods (3). The tubed biochemicals were incubated in air at 35°C for a maximum of 7 days. Standard tubed biochemicals were negative for esculin hydrolysis; the oxidation-fermentation of glucose, lactose, maltose, xylose, and sucrose; acetoin production; ornithine and lysine decarboxylation; citrate utilization; gelatinase; acetamide utilization; nitrate reduction; and the ability to grow in 6.5% NaCl. The lysozyme resistance test was determined by inoculating lysozyme broth (Remel) and a glycerol control broth. The isolate was lysozyme resistant. Urease results were variable. Arginine dihydrolase was positive. Both colony types were weakly catalase positive.

The RapID CB Plus enzyme strip (Remel, Lenexa, KS) was

FIG. 2. Lung biopsy tissue imprints of *Parastreptomyces abscessus* infection, showing predominantly coccoid gram-variable forms.

inoculated according to the manufacturer's specifications and incubated in air at 35°C for 4 h before the test results were recorded. Rapid enzyme tests (RapID CB Plus) gave bionumbers of 4407511 for the larger colony and 4407531 for the small colony, resulting in 98.3% and 99.9% confidence levels, respectively, for adequate identification of *Rhodococcus equi* (*Rhodococcus equi* is the only *Rhodococcus* species in the database). The colony types differed only in their ability to produce urease, which was produced by the smaller colony.

Susceptibility tests were performed by the gradient diffusion method (Etest; AB BIODISK North America, Inc., Piscataway, N.J.) according to the manufacturer's directions and with Mueller-Hinton agar plates. Susceptibility of the organism for selected antimicrobials was as follows: vancomycin, 0.75 µg/ml; rifampin, <0.002 µg/ml; penicillin, <0.002 µg/ml; and imipenem, 0.125 µg/ml.

Whole-cell fatty acid analysis was performed by gas-liquid chromatography using the Microbial Identification System (MIDI, Newark, DE) as previously described (7). The cells were grown on TSY (tryptic soy broth containing yeast extract) agar supplemented with 5% sheep blood at 35°C with a 5 to 10% CO₂ atmosphere and harvested at 5 days and 9 days. The extracted fatty acids were profiled by using a Hewlett-Packard 5890 Series II gas chromatograph with an electronic pulse control, a sample controller, and Vectra computer (Hewlett-Packard, Palo Alto CA) with MIDI's clinical library (version 4.0). The isolate did not perform well by this method; total areas were substandard. The library does not contain *Streptomyces* or *Brevibacterium* species, but it does contain *Arthrobacter*, *Rhodococcus*, and *Tsukamurella* species for comparison (6). The isolate was distinguished from *Rhodococcus* and *Tsukamurella* by the absence of tuberculostearic acid. It was distinguished from *Arthrobacter* by a significantly lower percentage of anteisopentadecanoic acid, which is the most prominent fatty acid in *Arthrobacter* cell walls; this peak is absent in *Rhodococcus* and *Tsukamurella*.

Genomic DNA was extracted from bacterial colonies by using the QIAamp DNA Mini kit (QIAGEN, Inc., Valencia, CA), and the 16S rRNA gene was PCR amplified and se-

FIG. 3. Neighbor-joining dendrogram based on 1,256 consecutive positions of the 16S rRNA gene with 1,000 bootstrap replications, showing the phylogenetic relationships of *Parastreptomyces abscessus* to species phenotypically related to the members of the order *Actinomycetales*. Numbers at each node correspond to the bootstrap values. GenBank accession numbers and strain numbers where appropriate are included.

quenced, both forward and reverse, as previously described (9). A 1,514-bp consensus sequence was generated and submitted to the GenBank database (DQ000673); the sequence was identical for both colony types. The consensus sequence was searched against GenBank with the BLAST tool (1). The 16S rRNA gene sequences from species phenotypically related to the members of the order *Actinomycetales* were retrieved from GenBank and were aligned with CLUSTAL_X software (11). A phylogenetic tree was constructed using PHYLIP, version 3.573 (J. Q. Felsenstein, Department of Genetics, University of Washington, Seattle, WA [http://evolution.genetics.washington.edu/phylip.html]) with *Arthrobacter* spp. used as the outgroup in the dendrogram. The branching order of the neighbor-joining dendrograms was evaluated with 1,000 bootstrap analyses by using the SEQBOOT program in the PHYLIP software package (5). The closest alignment to our isolate with DNA sequences in the GenBank database was quite distant: it shared only 94.9% sequence similarity with *Streptomyces cattleya* (1,411 out of 1,487 bp matching). The top 10 BLAST alignments were all *Streptomyces* species. The phylogenetic relationship of the patient isolate with the top 10 BLAST align-

ments and select GenBank isolates from three species in the family *Streptomycetaceae* (*Kitasatospora*, *Streptomyces*, and *Trichotomospora*) are shown in Fig. 3. The patient isolate aggregated in one clade containing the *Streptomyces*, *Kitasatospora*, and *Trichotomospora* species. The isolate was separated from these three genera with a high bootstrap value of 998.

Discussion. We have isolated an organism that was responsible for fatal pulmonary infection and bacteremia in an immunocompromised host. The clinical presentation of this infection bore many similarities to infections with *Rhodococcus equi*, which also causes pulmonary infections in transplant recipients and patients with human immunodeficiency virus/AIDS (12).

This organism is an asporogenous, nonmotile, gram-positive aerobe that grows slowly on all media. It demonstrated coccoid forms on direct Gram stains from clinical specimens and pleomorphic, striated coryne forms from both liquid and solid media. Tan colonies, 2 to 4 mm in diameter, grew on blood

agar after several days of incubation. It was weakly catalase positive, modified acid-fast negative, and relatively inert in standard biochemical tests. It grew best at 35°C in 5% CO₂ but not at all at 42°C. On tap water agar, it produced coccobacilli with diphtheroidal arrangements, which readily distinguished the isolate from *Streptomyces*. Further, gas-liquid chromatography analysis of esterified cellular fatty acids differentiated it from *Arthrobacter*, *Rhodococcus*, and *Tsukamurella*. The potentially unique nature of this organism was distinguished, however, by its novel 16S rDNA sequence. Based on these data and to facilitate future communications among clinicians and clinical laboratories, we propose the designation "*Parastreptomyces abscessus*" for this isolate.

Because this is the first described case of human infection with "*Parastreptomyces abscessus*" (and the first time the organism has been identified from the environment as well), its ecologic niche (and thus the source of our patient's exposure) is unknown. No epidemiologic clues were identified—our patient did not have any recent exposure to rendered soil, animals, or farms and indeed was spending most of his time in the outpatient department of a hospital at the time he contracted the illness. Given his primary pulmonary presentation, acquisition was likely via inhalation, which may imply that the organism is an environmental saprophyte akin to *Rhodococcus* or *Streptomyces* spp.

Clinically, infection with this isolate in a severely immunocompromised host closely resembled infection with *Rhodococcus equi*. As was the case with our patient, most infections with *Rhodococcus* in transplant recipients involve the lung, where intrahistiocytic localization of infection causes granulomatous inflammation (10). With macrophage destruction, local expansion of infection results in purulent necrosis. For these reasons, many believe that antimicrobial therapy of *R. equi* infections should include agents with good intracellular penetration (12). Despite the use of multiple antimicrobials (including those with and without intracellular penetration) to which the organism was susceptible, our patient died from rapidly progressive pulmonary infection. This aggressive clinical course was likely due to the intense immunosuppression the patient had received, in addition to the extent of pulmonary infection prior to the addition of penicillin and rifampin therapy, rather than to the inherent pathogenicity of the organism. Nevertheless, this report illustrates that the use of ever-more-aggressive immu-

nosuppressive regimens in stem cell and solid-organ transplant recipients will herald new infections with both described and previously undescribed saprophytic microbes.

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ADDENDUM IN PROOF

After submission of the manuscript, we recovered another isolate of "*Parastreptomyces abscessus*" from the blood of a 65-year-old patient about 1 year after kidney transplant. This patient had been experiencing several days of chills, fever, and cough while on immunosuppressive therapy (tacrolimus [inhibits interleukin-2 and interleukin-2 receptor production and blocks cell division] and prednisone) and was diagnosed with acute pneumonia complicated by chronic obstructive pulmonary disease.

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